

## Single Molecule Techniques II

### 2638-Pos Board B657

#### Mechanisms in Co-Translational Protein Folding Elucidated using Single Molecule FRET

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In nature, protein folding mechanisms are determined by both amino acid sequence AND physiological context. Inside living cells the transient and vectorial nature of protein synthesis, the presence of molecular chaperones, and macromolecular crowding can each have a profound effect on the paths nascent chains take en route to their native states. This adds a daunting level of complexity to the study of *in-vivo* protein folding mechanisms. To begin to address these issues we have developed methods which enable the detection of ribosome-bound nascent chain (RNC) conformational distributions and dynamics using single molecule fluorescence resonance energy transfer. Our approach employs a purified and reconstituted *E. coli* *in-vitro* translation system to generate stalled RNCs, the site-specific incorporation of chemically-reactive alkyne-bearing unnatural amino acid tags into nascent chains, and post-translational bioconjugation of pairs of single-molecule donor and acceptor fluorophores to the RNCs using a highly-efficient ligand accelerated copper-click chemistry reaction. We apply our novel RNC labeling approach to explore the role of helix stabilization by and propagation from the exit tunnel of the ribosome as a general means by which the ribosome may direct the co-translational folding of nascent polypeptides.



### 2639-Pos Board B658

#### Learning Kinetic Models from Single-Molecule FRET Data using Bayesian Inference

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Single-molecule FRET studies have enabled real-time observation of conformational transitions in individual molecules, allowing targeted investigations into the mechanistic function of molecular machines such as the ribosome. Like in many single-molecule platforms, a fundamental problem with sm-FRET studies is that our noisy fluorescence signal does not unambiguously determine the underlying conformational state. Moreover, a single experiment often yields hundreds of time series, which report on the same underlying process, but exhibit significant variations in photophysical properties and kinetic rates. This combination of lots of data and lots of stochasticity means that interpretation of sm-FRET experiments often requires use of statistical inference techniques. Hidden Markov Models are a widely used tool for parameter estimation in time series data, and have been successfully applied to sm-FRET experiments by several groups. A fundamental limitation of existing approaches is that inference is only performed on one time series at a time, yielding a large number of parameter estimates of variable quality which must now be related to each other using ad-hoc experiment specific post-processing steps.

Here, we propose a technique known in the statistical community as Empirical Bayes estimation, to perform combined analysis on the entire collection of trajectories in an experiment. This allows straightforward and statistically principled learning of a consensus kinetic model from an ensemble of time series. Moreover, the method allows significantly better estimates of the kinetic rates associated with conformational transitions. Finally we demonstrate how inference results on models with varying kinetic structures can be compared to directly test detailed mechanistic hypotheses in a statistically principled, adaptable manner.

### 2640-Pos Board B659

#### Single-Molecule FRET Studies of a Y-family Polymerase Provide New Insights into Nucleotide Binding Mechanism

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High fidelity polymerases are normally blocked by damage in the DNA template. Stalled replisomes can lead to DNA double-strand breaks or other detrimental genotoxic effects, thereby increasing genome instability. A specialized variety of DNA-polymerases is mobilized when replication across damaged bases (translesion DNA synthesis) is required. Y-family polymerases perform the majority of translesion synthesis and are generally found to be specialized for

specific types of lesions. These Y-family polymerases typically present a wide active site to accommodate distorted/bulky DNA.

Dpo4 is a Y-family polymerase that has been extensively characterized by ensemble experiments. However, the exact polymerization mechanism that occurs during the bypass of the DNA damage remains unclear. We have used single-molecule fluorescence resonance energy transfer (smFRET) to investigate the interactions between DNA and Dpo4. Our data show that Dpo4 binds DNA in two different conformations that interconvert reversibly. Experiments carried out in the presence of nucleotides using  $\text{Ca}^{2+}$  instead of  $\text{Mg}^{2+}$  to prevent DNA extension suggest that one of these conformations is preordered to accept the incoming nucleotide. We were able to characterize the binding and dissociation dynamics between nucleotides and the DNA/Dpo4 complex. Our results also suggest that Dpo4 undergoes a similar conformational rearrangement upon binding a correct or incorrect (mismatched) nucleotide.

### 2641-Pos Board B660

#### Internucleosomal Interactions Monitored at a Single Molecule Level

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We studied interactions between two nucleosomes at a single molecule level using FRET. According to the results, internucleosomal interactions strongly depend on various histone modifications. Our unique single molecule setup will facilitate investigations on the detailed structure of chromatin packaging affected by histone modifications.

### 2642-Pos Board B661

#### Simple Autofocusing System for Single-Molecule FRET Experiment Based on Single-Molecule Image Analysis

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Single-molecule FRET has greatly contributed to our detailed mechanistic understanding of many bio-molecular systems. While reactions occurring in the range of several minutes can be readily studied, data acquisition for longer time scales is hindered by accumulated focal drift of a high numerical aperture objective, which should be corrected in real time. Here, we develop an autofocusing system based on the analysis of optical astigmatism of single-fluorophore images. Compared to the other autofocusing methods, our approach has a merit of simplicity that neither fiducial markers nor additional light sources and detectors are required. As a demonstration of the new autofocusing system, we observed slow B-Z transition dynamics occurring in several hours using single molecule FRET.

### 2643-Pos Board B662

#### Unfolding and Degradation of Proteins by ClpXP Monitored with Single Molecule FRET

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Proteins, the working machineries inside a cell, are tightly regulated from birth through maturation to death. ClpXP is an ATP dependent protease that is involved in protein quality control and regulation. ClpX is a ring-shaped ATPase that recognizes, denatures and translocates target substrates into ClpP. ClpP is a barrel shaped protease that degrades polypeptide substrates. Despite extensive biochemical studies over the last two decades, much remains unknown about the molecular mechanism of ClpXP. We will visualize the whole process of substrate recognition, denaturation, translocation, and degradation using multicolor single-molecule FRET. This single-molecule study will reveal the mechanism of degradation and product release by ClpXP.

### 2644-Pos Board B663

#### High Speed Magnetic Tweezers at 100KHz with Superluminescent Diode Illumination

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Magnetic tweezers apply force to single DNA molecules to measure changes in DNA length as a function of time. The technique is ideally suited to explore the dynamics of biophysical processes such as DNA unzipping by helicase motor proteins due to the constant force applied to the DNA. However, instrumental improvements are needed to resolve the length and time scales of single enzymatic steps. Here, we introduce a superluminescent diode as the illumination source and a high-speed CMOS camera as the optical detector to achieve magnetic tweezing of DNA at a bandwidth of 100 kHz. We show how GPU-accelerated video processing can be used to determine three-dimensional particle positions from a series of video frames with a spatial resolution below 1 Angstrom. We demonstrate the quantitative capabilities of this instrument by measuring the drag coefficient of a magnetic bead and the stiffness of a tethered DNA molecule at a corner-frequency of